Design of Bacillus anthracis Lethal Factor Protein Inhibitor Through Structure-Based Design

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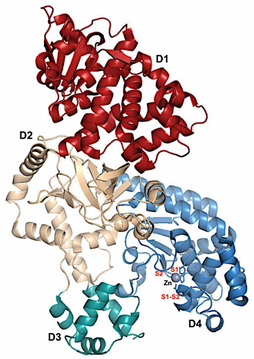
I. Abstract

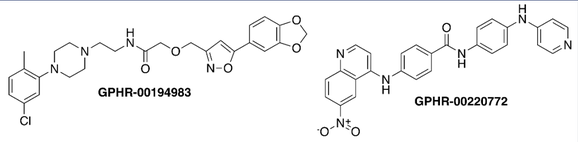
Anthrax is an infection caused by *Bacillus anthracis*, a bacterial species that produces a deadly toxin.

This toxin is composed of three proteins: protective antigen (PA), edema factor (EF), and lethal factor (LF). Currently, there are no commercial treatments for anthrax-induced toxemia. Available treatments include antibiotics, which are only effective in early disease stages and have no effect on the toxin once its produced, or antibodies, which have their own pharmacokinetic liabilities.[1] LF is the primary target of this study, and the goal was to design a small-molecule inhibitor using a structure-based design.

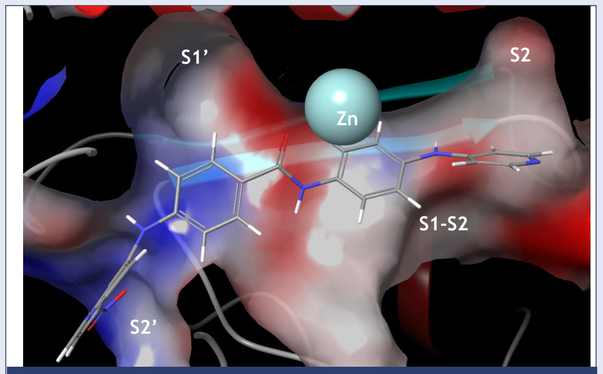


During this iterative design process, the structural components of our compounds were modified to see how this affected the affinity of these compounds as predicted by Glide. Compounds with more favorable docking scores were retained and modified, and compounds with less favorable docking scores were discarded. So far, optimization of the S1' pocket and S2 is underway. Here we present the compounds designed based upon many rational design trials.

II. Objective

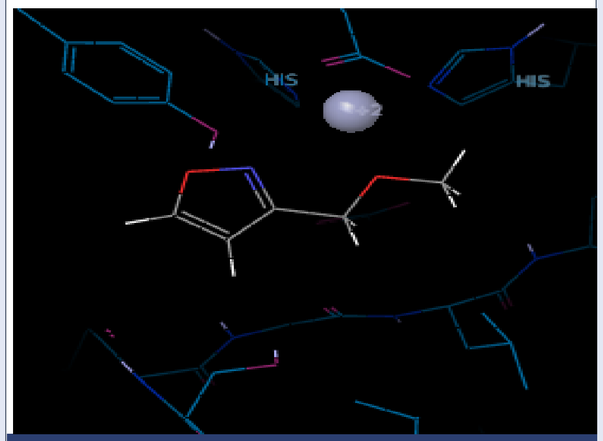


In order to design a new inhibitor against LF, a structure-based design was used. GPHR-00194983 was used as a starting point since it was previously identified as an inhibitor of LF from a library of approximately 230,000;[2] however, due to its solubility, it was difficult to perform co-crystallization studies with LF. For this reason, a new compound had to be designed and synthesized, hopefully with improved solubility, more favorable pharmacokinetic profiles and similar affinity for the LF active site. Using the isoxazole core of **GPHR-00194983** as a starting point, different parts of the molecule could be designed to interact with each pocket of the active site, as well as the catalytic zinc atom. By optimizing the interactions between the designed compounds and each pocket within the active site individually, we hoped to design a molecule with better binding and solubility than **GPHR-00194983.** Compounds were docked into the LF active site (PDB:1YQY)[3] using Glide in Maestro.[4,5]



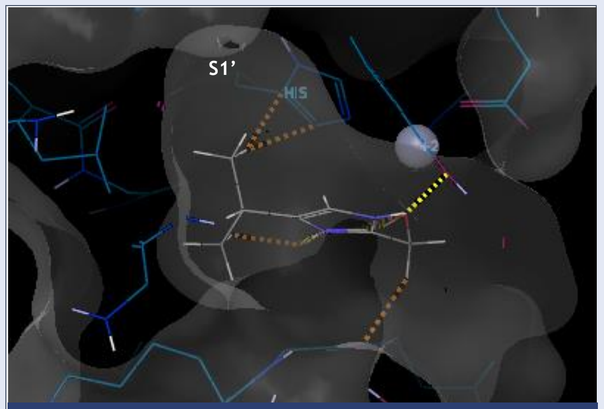
III. Optimization of the Core

The isoxazole core of **GPHR-00194983 was used as a starting point for the design process. This core was tested with different side chains to see how they would fit into the active site. It was observed that an isoxazole ring with a dimethyl ether side chain coordinates with the zinc atom via the isoxazole nitrogen and the ether oxygen. Further testing showed that an imidazole ring bound more consistently in this orientation than the isoxazole core. This core would further be modified with an "R1" group directly attached to the core, and an "R2" group attached to the terminal methyl group.**



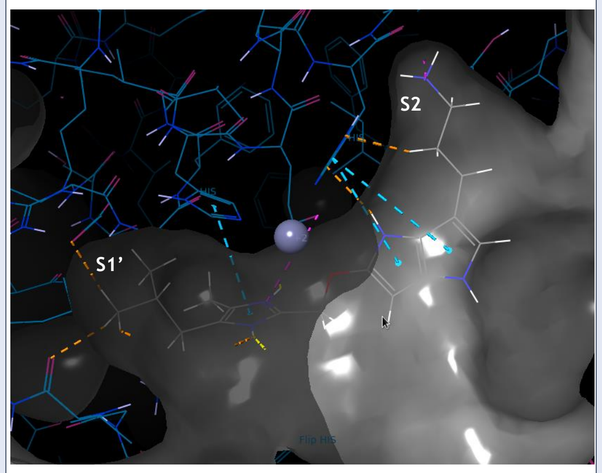
IV. Optimization of "R1"

The R1 group was designed to interact with the S1' pocket. First, a variety of substituents were tested, varying from halogens to ethers to hydrophobic groups. It was determined that the hydrophobic groups participated in beneficial interactions with the pocket and docked into the S1' pocket consistently. It was thought that these hydrophobic groups were the best fit, but later tests included amine groups in order to test hydrogen bonding. These experiments showed that the amino acids near the S1' pocket were able to accept hydrogen bonds. This meant that a terminal amine group included in the R1 was predicted to have equal or higher affinity than the strictly hydrophobic groups.



V. Optimization of "R2"

The next part of the design process consisted of finding a group capable of reaching the S2 pocket of the active site. The initial problem with the design of this group was that the S2 pocket is far away from where the core was predicted to bind. This meant that if the R2 group was able to freely rotate, there would be little probability that the group would be docked into the pocket. To fix this issue, it was determined that a bicyclic ring structure would help the compounds reach the pocket. Since these rings are constrained, they were able to better reach the S2 pocket. We then determined that an alkyl chain ending with a terminal amine fit well into the pocket since it can act as a hydrogen bond donor. Depending on the ring's structure, a carbon chain of 2-3 carbons worked best to attach the amine to the bicyclic. Although the bicyclic ring systems used during the initial iterations of the design process worked well in terms of docking, we realized that the resulting compounds were not synthetically feasible. The most recent iterations of our design consisted of testing different bicyclic rings to hopefully find something more synthesizable.



VI. Conclusions

Overall, 19 iterations of the rational design process were performed. Compounds based upon **GPHR-00194983** were designed, and their binding poses were modeled using Glide. Through our iterative structure-based design process, we have designed a library of compounds with more favorable docking scores than the original compound. At this point in time, the compounds in our library are optimized for interactions with the S1' pocket and the zinc atom, and future studies will finish the optimization of the S2 pocket. The main goal of this project was to design an inhibitor for LF. While the optimization is not finished, compounds have been designed that have greater affinity for LF than **GPHR-00194983**. The second goal of this project was to synthesize these compounds and determine their IC_{50} values. This objective was not completed since the compounds proved to be too difficult to synthesize. The project's time frame ended while the compounds were being redesigned for synthesizability.

VII. Future Work

Future work includes finishing the optimization of the designed compounds. After this, we plan on synthesizing some of the compounds and testing their inhibition against LF. Eventually, once a compound with biological activity against LF is found, co-crystallization studies will be performed.

VIII. Acknowledgments

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